



## SOIL & CROP SCIENCES | RESEARCH ARTICLE

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# Does zinc biofortification affects the antioxidant activity in common bean?

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**Abstract:** The purpose of this work was to assess the consequence of zinc biofortification on zinc (Zn) content, natural processes of hydrogen peroxide-scavenging enzymes, and biomass production of green bean (*Phaseolus vulgaris* L.) under greenhouse conditions. Zn was applied using two forms (ZnSO<sub>4</sub> and DTPA-Zn) at four doses of application (0, 25, 50 and 100 μM) added under a hydroponic system, and were tested over a period of 60 days. The Zn content was assessed in seeds, as well as the activity of antioxidant enzymes, and hydrogen peroxide production. The results showed that application of low-dose zinc increased concentration in the seed, regardless of the form of application. Respect to antioxidant enzymes, dose of 25 μM of Zn significantly increased activity of the enzyme superoxide dismutase. Finally, to raise the Zn concentration in bean under biofortification program was a promising strategy in cropping systems in order to increase the ingestion of iron and antioxidant capacity in the general population and provided the benefits that this element offered in human health.

**Subjects:** Environment & Agriculture; Agriculture & Environmental Sciences; Food Science & Technology

**Keywords:** antioxidant activity; zinc; *Phaseolus vulgaris*; catalase; superoxide dismutase; mineral malnutrition

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### PUBLIC INTEREST STATEMENT

Zinc deficiency affects 30% of world population. There are several effects of zinc deficiency such as growth in children, brain disorders, high cholesterol, female reproduction, etc. Common bean is a staple food crop and is the major source of iron, zinc and protein in Africa and Latin America. Micronutrients can be increased in beans through biofortification, that is the increase in the concentration of nutrients in food crops through the application of fertilizers to soil or leaves. There are other types of biofortification, such as genetic and biotechnological, but the use of fertilizers (agronomic biofortification) is the cost-effective way to increase nutritional quality in plants. In this study, we found that zinc biofortification in common bean can improve the concentration of this element in human diet at low cost, especially in developing countries. Incidence of several diseases related with Zn malnutrition can be reduced by the consumption of biofortified beans.

## 1. Introduction

Zinc (Zn) is an important transitional metal, and is the only metal present in all six classes of enzymes and act as component for several transcriptional factors (Prasad et al., 2012). Zn plays an important role in the synthesis of proteins and carbohydrates (Sajedi, Ardakani, Naderi, Madani, & Boojar, 2009).

Several research reports have established the essentiality and role of micronutrient-zinc on plant growth, development and yield (Grzebisz, Wronska, Diatta, & Dullin, 1999). Zn is the second most abundant transition metal after iron and is involved in various biological processes in organisms.

Nevertheless, it is not clear what the implications of Zn stress are on antioxidant responses and the intake of nutrition (Wang et al., 2009). To reduce stress, plants produce antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), to keep the ROS lower than the toxic limit (Gill et al., 2015). Soil and foliar biofortification with Zn is considered an effective short-term solution to Zn deficiency-associated troubles in both crop production and human health (Manzeke, Mtambanengwe, Nezomba, & Mapfumo, 2014). However, there is little information about the way in which Zn biofortification can influence the nutritional quality of plants and particularly the antioxidant capacity. Therefore, the purpose of the present work was to examine the effect of Zn biofortification with different application rates and forms of Zn, on the antioxidant capacity and nutritional quality of the bean as bioindicators of an efficient Zn beautification program in bean plants (*Phaseolus vulgaris* L.).

## 2. Experimental

Seeds of bean (*Phaseolus vulgaris* L., cv. Strike) were germinated and grown in a substrate mix (peat moss, vermiculite and perlite at a ratio 3:1:1) at an experimental greenhouse located in Delicias, Chihuahua, Mexico. The temperature inside of the greenhouse was controlled at  $25 \pm 4^\circ\text{C}$ . Plants were grown in individual pots (25 cm upper diameter, 17 cm lower diameter, 25 cm in height) of the 8 L volume, filled with substrate mix. There was used four plants per pot. Throughout the growing cycle the bean plants received a growth, nutrient solution (Hoagland & Arnon, 1950). Zn treatments were used in combination with the nutrient solution for the last 40 days. The first 20 days after germination, it was used only the nutrient solution. In this study was utilized a completely randomized experimental design, with different forms of Zn at different concentrations. This was four treatments of Zn chelate, at four doses and four replicates per treatment (one pot was one replicate): DTPA-Zn and  $\text{ZnSO}_4$  at doses of 0, 25, 50 and 100  $\mu\text{M}$ , respectively.

Plant materials were sampled at 60 days after germination, when the plants had a phenological phase of complete development and fruit maturity. One part of plant material (plant fresh matter) was frozen using liquid nitrogen and stored at  $-30^\circ\text{C}$ , this material was used for antioxidant enzyme assays (SOD, CAT, APX and GSH-PX) and  $\text{H}_2\text{O}_2$  quantification. The rest of the plant material (plant dry matter) was dried at  $65^\circ\text{C}$  and used to determine the biomass, and Zn concentration in bean seed. Plant biomass were determined as the average dry weight of the entire plant and expressed as  $\text{mg DW}^{-1}$ . Zn concentration was determined by an Induced Plasma Optical Emission Spectrometer (Agilent Technologies 700 Series ICP-OES, California, USA), according to the method described by Karacan and Aslantaş (2008). Zn concentration was expressed in  $\text{mg kg}^{-1}$  of dry weight.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was defined by its power to suppress the formation of nitroblueformazan from NBT according to Giannopolitis and Ries (1977). Catalase (CAT) (EC 1.11.1.6) activity was determined by spectrophotometrically following  $\text{H}_2\text{O}_2$  consumption at 240 nm (Rao, Paliyath, Ormrod, Murr, & Watkins, 1997). Glutathione peroxidase (GSH-PX) (EC 1.11.1.9) activity was measured using  $\text{H}_2\text{O}_2$  as substrate (Flohé & Günzler, 1984).

Hydrogen peroxide content of seed samples was colorimetrically measured (Brennan & Frenkel, 1977).  $\text{H}_2\text{O}_2$  concentration was calculated according to (Sánchez et al., 2000).

Data were subjected to a simple ANOVA at 95% confidence, using SAS (SAS Institute Inc., Cary, NC). Means were compared by Tukey test ( $p \leq 0.05$ ). The data shown are mean values  $\pm$  standard error (SE).

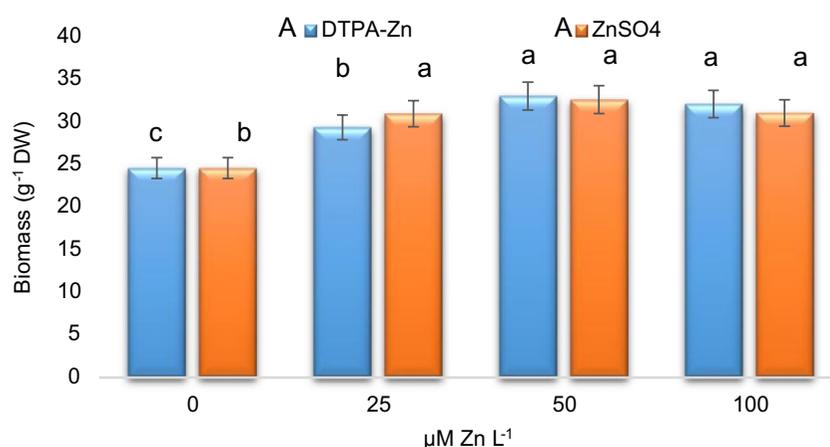
### 3. Results and discussion

It was found that zinc application to crops enhanced biomass production and plant zinc concentration (Subbaiah et al., 2016). Our results agree with those obtained, showing a significant increase with respect to the control in plant biomass and Zn content in the seed at low doses of Zn (Figures 1 and 2). One of the most widely used indicators of stress is plant biomass (Ríos et al., 2009). Therefore, we studied this indicator of stress as a one way to define the effect of the different Zn treatments and we observed that the different doses and forms of Zn applied were significantly different from the control. Respect to Zn accumulation in seed, Zn biofortification induced the accumulation of this mineral in bean seed, with the two Zn forms applied, being the most convenient doses of 25  $\mu\text{M}$ , as no significant differences were observed between doses (Figure 2 and Table 2).

$\text{H}_2\text{O}_2$  accumulation is one of the causes of lipid peroxidation that affects lipid membranes (Djanaguiraman, Devi, Shanker, Sheeba, & Bangarusamy, 2005).  $\text{H}_2\text{O}_2$  is relatively “safe” in the absence of transition metals, being unreactive even at levels higher than biological system would ever

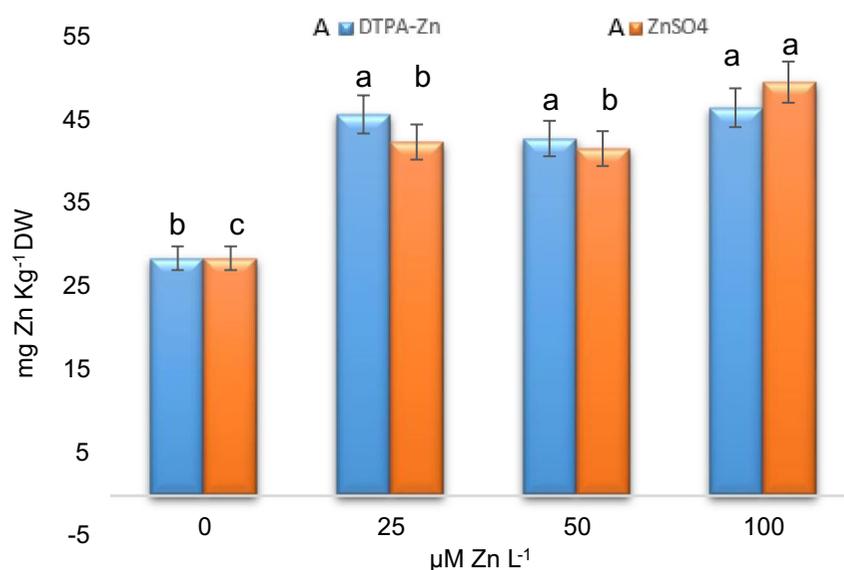
**Figure 1. Biomass in plants of *P. vulgaris* L. cv Strike subjected to different zinc doses and forms.**

Notes: Data are means  $\pm$  SE ( $n = 4$ ) ( $p \leq 0.05$ ). Means with the same letter are not significantly different.



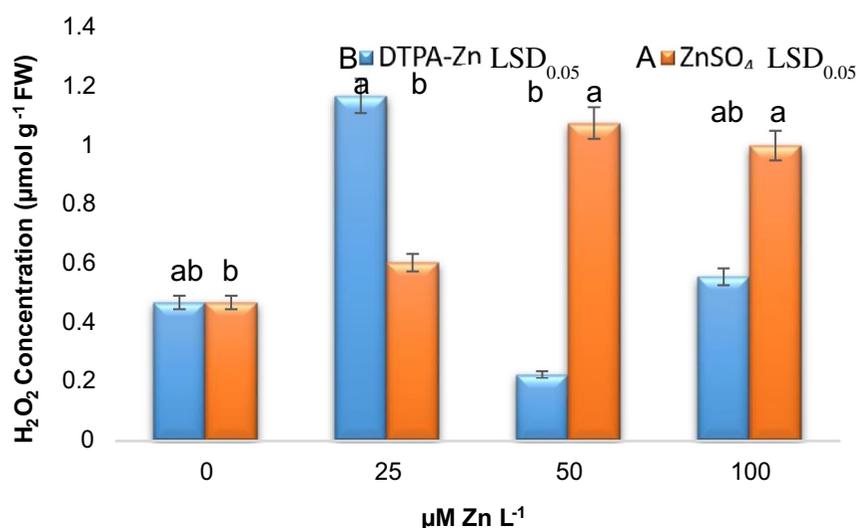
**Figure 2. Zn concentration in bean seeds subjected to different concentrations and forms of Zn.**

Notes: Data are means  $\pm$  SE ( $n = 4$ ) ( $p \leq 0.05$ ). Means with the same letter are not significantly different.



**Figure 3. Hydrogen peroxide activity in seeds of *P. vulgaris* L. cv Strike subjected to different zinc doses and forms.**

Notes: Data are means ± SE (n = 4) (p ≤ 0.05). Means with the same letter are not significantly different.



generate. In previous studies of Se biofortification, it has been found that the content of H<sub>2</sub>O<sub>2</sub> in seed can increase as increasing the doses of Se, which can be toxic levels and produce oxidative stress (Ríos et al., 2009). In this study, our results showed significant differences with DTPA-Zn, respect to the control, where 50 µM was the lowest (Figure 3). For these reasons, we assumed that DTPA-Zn is less toxic than ZnSO<sub>4</sub> in this bean crop.

Improvement of stress tolerance to toxic levels of metals is often linked to an increase in activity of antioxidant enzymes. Actually, early accumulation of H<sub>2</sub>O<sub>2</sub> during metal exposition can result in an increase in antioxidant enzyme activities, which in turn protect plants from oxidative stress caused by metals such as Zn. Therefore, increased SOD activity in bean seeds indicate that this specie of plant has the capacity to adjust to high levels of ROS by developing an antioxidant defense system. SOD is an important member of the cell protective antioxidant system. This enzyme catalyzes the dismutation of the superoxide anion into H<sub>2</sub>O<sub>2</sub> plus molecular oxygen (Ślesak, Ślesak, Libik, & Miszalski, 2008). We found that SOD activity significantly increased in bean seeds as lower DTPA-Zn dose was used (25 µM) (Table 1). These increased resulted highly significant with respect to the control. The high activity of SOD may be due to a high concentration of superoxide radicals, which in turn are reflected in the high concentration of hydrogen peroxide in this form of application (DTPA-Zn 25 µM) (Figure 3).

**Table 1. CAT, SOD and GSH-Px activities in bean seeds to different concentrations and forms of Zn**

Treatments	CAT (µmol H <sub>2</sub> O <sub>2</sub> min <sup>-1</sup> g <sup>-1</sup> FW)		SOD (U min <sup>-1</sup> g <sup>-1</sup> FW)		GSH-Px (nmol GSH min <sup>-1</sup> g <sup>-1</sup> FW)	
	Zn-DTPA	ZnSO <sub>4</sub>	Zn-DTPA	ZnSO <sub>4</sub>	Zn-DTPA	ZnSO <sub>4</sub>
0	0.84 ± 0.03 a	0.84 ± 0.03 a	1.94 ± 0.11 c	1.94 ± 0.11 c	3.03 ± 0.05 a	3.03 ± 0.05 a
25	0.89 ± 0.02 a	0.96 ± 0.32 a	3.66 ± 0.02 a	3.61 ± 0.03 a	2.89 ± 0.04 a	2.70 ± 0.05 ab
50	1.67 ± 0.02 a	0.91 ± 0.10 a	2.44 ± 0.19 b	2.74 ± 0.29 b	3.48 ± 0.41 a	2.42 ± 0.05 b
100	1.76 ± 0.46 a	1.12 ± 0.20 a	2.16 ± 0.02 bc	1.39 ± 0.06 c	3.21 ± 0.22 a	1.85 ± 0.20 c

Notes: CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; FW, fresh weight; Data are means ± SE. Means followed by different letters in each column are significant at p < 0.05 (n = 4).

**Table 2. Statistical summary showing main effects and interactions for biomass, Zn content, CAT, SOD, GSH-Px and H<sub>2</sub>O<sub>2</sub>**

Source	Biomass	Zn content	CAT	SOD	GSH-Px	H <sub>2</sub> O <sub>2</sub>
Treatment	NS	NS	*	NS	***	*
Dose	***	***	*	***	*	*
Treat × Dose	NS	*	NS	**	**	***

Note: NS = No significant.

\**p* < 0.05.

\*\**p* < 0.01.

\*\*\**p* < 0.001.

#### 4. Conclusions

Zn Biofortification increased significantly the accumulation of Zn in bean seeds, we considered that the best treatment is the application of DTPA-Zn at 25 μM dose, which increased in the high percentage levels of Zn in bean seeds and antioxidant activity of this crop. In general, these results will contribute to defining the utility and application of Zn biofortification, which promote the application of micronutrients at low rates. This technology has a great potential to control the induction of the antioxidant system in bean plants, thereby improving crop yield, stress resistance and accumulation of antioxidant compounds in bean seeds. Factor with more influence was dose, with significance on all variables. Interaction had influence on Zn content, two enzymes and hydrogen peroxide. Fertilizer used had effect on CAT, GSH-Px and hydrogen peroxide.

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#### Competing Interests

The authors declare no competing interest.

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#### Cover image

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